

# In-Situ Electrochemical Generation and Utilization of Hydrogen Peroxide for Disinfection

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**Disinfection needs to meet hygiene requirements at the International Space Station (ISS) is currently accomplished through the use of pre-packaged, disposable, wetted wipes, which represent an appreciable carry-along mass and disposal burden. However, as human missions travel further into the solar system the availability of resources to resupply will be diminished. Therefore, next-generation systems should use onboard utilities to create on demand disinfectants, thereby reducing the dependence on earth-based supplies and further eliminating storage and disposal problems. Accordingly, we are developing an *in-situ* approach to electrochemically generate hydrogen peroxide disinfecting solution utilizing onboard life support supplies (Air/Water) to neutralize surface microorganisms present in closed living systems. As discussed within our 2019 and 2021 ICES papers (ICES-2019-38 and ICES-2021-273), we have continued to improve our technology readiness level by scaling the electrochemical generation production process and validating the system in a zero-gravity parabolic flight test. In this paper we will demonstrate a system that can achieve over 1 L of >2 w/w% peroxide per day with deionized water and air feeds. The hydrogen peroxide solutions generated were sent to NASA for microbial control property characterization. Overall, the electrochemical peroxide generation system offers a more economical and practical alternative, with the disinfectant being generated on demand and *in-situ* (using**

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available life support materials of Air/Water); and applied to reusable cloths. The specific application of interest to this program is crew contact surfaces in space vehicles, but this technology could be utilized for waste water disinfection, heat exchanger biofouling remediation, and laundry applications. The peroxide generation system will also be able to address Earth-based needs in various settings such as field hospitals, restaurants, military, warehouses, movie theatres, among many others.

## Nomenclature

<i>AEM</i>	=	Anion Exchange Membrane
<i>ANOVA</i>	=	Analysis of variance
<i>BPR</i>	=	Back Pressure Regulator
<i>CDC</i>	=	Centers for Disease Control and Prevention
<i>DI</i>	=	Deionized
<i>EML</i>	=	Electrochemical Microgravity Laboratory
<i>EEB</i>	=	Environmental Enclosure Box
<i>GDE</i>	=	Gas Diffusion Electrode
<i>ICES</i>	=	International Conference on Environmental Systems
<i>ISS</i>	=	International Space Station
<i>MPL</i>	=	MicroPorous Layer
<i>MMO</i>	=	Mixed Metal Oxide
<i>PGU</i>	=	Peroxide Generation Unit
<i>PEM</i>	=	Proton Exchange Membrane
<i>PEEK</i>	=	Polyether Ether Ketone
<i>PVC</i>	=	Polyvinyl Chloride
<i>RO</i>	=	Reverse Osmosis
<i>SCC</i>	=	Safety Containment Chambers
<i>TBD</i>	=	To Be Determined
<i>UPR</i>	=	University of Puerto Rico
<i>UV-Vis</i>	=	Ultraviolet-visible
<i>w/w%</i>	=	Weight Percent

## I. Introduction

Solutions and innovations are needed to facilitate long duration and crewed deep space missions. One essential technological innovation is desired to meet the hygiene (disinfection) requirements of astronauts. Currently, the disinfection needs of astronauts at the International Space Station (ISS) are accomplished through wetted pre-packaged disposable wipes; where a 6 g wet wipe contains about 5 g of 0.5 w/w% hydrogen peroxide solution (Figure 1). The disinfectant wipe usage rate on ISS is on an average 3.8 wipes/person/day (equivalent to 8 kg of these wipes per person per year), which represents an appreciable carry-along mass and disposal burden. Accordingly, “a mechanism for the in-situ generation of cleaning/sanitizing solutions is needed that will enable these solutions to be applied to reusable fiber-based wipes to remove particulate, food, and body oil soiling of surface.”<sup>1</sup> Disinfection solutions produced by such an innovation must serve an anti-microbial function, demonstrating effectiveness against fecal coliform bacteria, food based bacteria, and iodine resistant bacteria.<sup>1</sup> Moreover, the disinfection solutions should be non-hazardous due to direct crew contact and direct off-gassing and accumulation of solutions in cabin atmosphere. Such *in-situ* generation of disinfection solutions will replace the existing consumables-intensive approach. Accordingly, as introduced in our prior work<sup>2</sup> and 2019 and 2021 ICES paper (ICES-2019-38 and ICES-2021-273),<sup>3,4</sup> Faraday Technology Inc. (Englewood, OH) is developing a system for *in situ* generation of hydrogen peroxide ( $H_2O_2$ ) utilizing on-board life support components of air and water.



**Figure 1. Hygiene towel for hydration and wetted wipes for surface disinfection currently used by astronauts at the ISS**

As described in previous publications,<sup>2,3,4</sup> hydrogen peroxide is electrochemically produced by reduction of O<sub>2</sub> ( $O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$ ) in presence of an aqueous electrolyte (water), has non-toxic decomposition products (viz., O<sub>2</sub> and H<sub>2</sub>O), is safe for direct skin contact at functionally active concentrations (~1-5 w/w%), and has a demonstrated disinfection capability.<sup>5</sup> Hydrogen peroxide is thus an ideal disinfection solution in the enclosed space vehicle crew habitat. In the activity described in this paper, the Faraday and NASA team: 1) identified potential concentrations and quantities of hydrogen peroxide desired for various applications; 2) designed, built, and optimized an alpha scale system that can produce up to 1 L/day of ~3 w/w% hydrogen peroxide; and 3) evaluated the generated peroxide's capability for microbial control.

## II. Development

### A. Potential Uses of Hydrogen Peroxide Generated in Space (including ISS disinfectant wipes)

Several uses of hydrogen peroxide on long endurance space missions have been envisioned. As the possibility of *in-situ* generation and utilization came closer through this SBIR technology development, experts in life support, medical and habitation systems were surveyed to discover their potential requirements. Results of the survey are shown in Table 1.

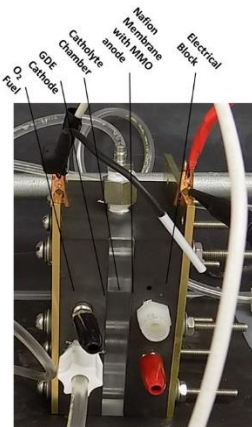
**Table 1. Potential Uses of Hydrogen Peroxide in Space Exploration Missions**

System	Application	Is 3 w/w% H <sub>2</sub> O <sub>2</sub> adequate?	Quantity Needed for a crew of 4 (ml)	Need Interval
Habitation	Disinfecting wipes	Y	525	regular - weekly
Clothes Cleaning	Stain treatment	Y	25	occasional
Exploration Medical	Wound care	Y	120	occasional
Exploration Medical	Equipment disinfection	Y	120	occasional
Plant Growth	Equipment sanitization	Y	300 ml once every 28 days	regular - monthly
Class D Plant Chambers	Biofilm prevention in payload subsystems	Y	200 ml once every 150 days	regular
Water	Urine pretreatment	Y	16.5ml/flush*7flush/day*4crew=460 mL/day	regular - daily
Water	Wastewater biocide	Y	TBD	TBD
Water - Bioreactors	Biofilm prevention; equipment sanitization of bioreactors	Y	300 ml+ once every 50 days	regular
Planetary Protection	Cleaning tools and instruments used to collect and study Mars material	TBD	TBD	TBD

Based on the results of the survey, a target concentration of 3 w/w% hydrogen peroxide was selected. Some uses, such as for plant growth systems, would be infrequent but predicable. Thus, the desired quantity could be generated over several days. The largest potential daily use is for urine pretreatment, which could require almost a half-liter per day. Another likely regular use, disinfecting wipes for weekly surface cleaning, was estimated to need 525 ml of 3 w/w% solution. The survey confirmed that the SBIR target production rate of 1 liter/day should be sufficient to serve multiple users.

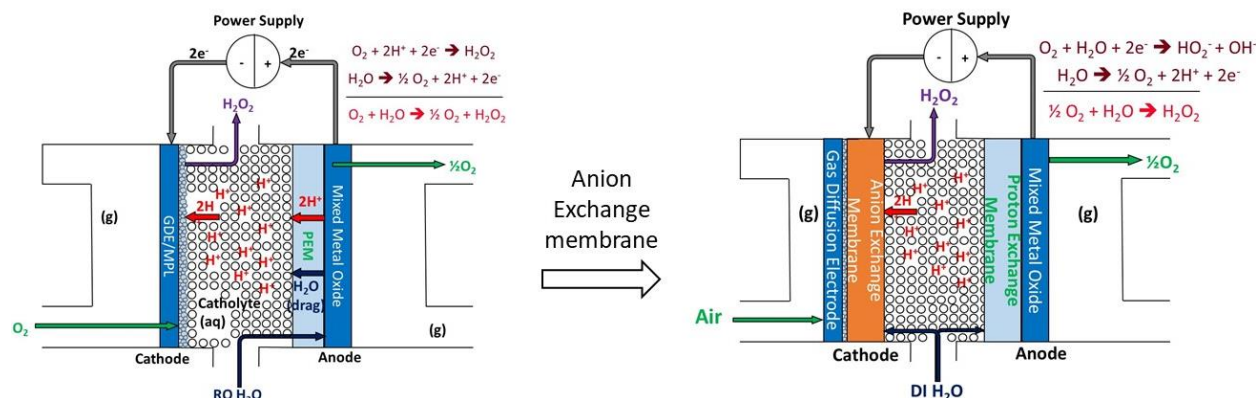
## B. Peroxide Generation Unit

A sub-scale peroxide generation unit (PGU; shown in Figure 2) with 6.25 cm<sup>2</sup> active electrode area was designed/built/optimized at Faraday and evaluated in a low-gravity flight test with reverse osmosis (RO) treated water and oxygen. These results were presented in our ICES 2019 and 2021 manuscripts.<sup>3,4</sup> The low-gravity flight test demonstrated that there were no adverse effects of zero-gravity on the performance of PGU in comparison to ground operation. The following are the components of the optimized PGU: 1) ion exchange resin consisting of Amberlite IR120; 2) a gas diffusion electrode (GDE) cathode consisting of CeTech WIS1005; 3) catholyte consisting of water treated with reverse osmosis (RO) process; 4) a PEEK catholyte chamber with 0.37" thickness; 5) a titanium anode chamber; 6) an expanded mesh dimensionally stable anode welded into the Ti anode chamber; 7) a graphite cathode chamber; 8) an oxygen gas feed in the cathode chamber; 9) a back pressure balance manifold (Equilibar LF Series Precision Back Pressure Regulator) around GDE to eliminate catholyte feed into the cathode chamber; and 10) a Nafion 117 proton exchange membrane (PEM).



**Figure 2. Sub-scale peroxide generation unit (PGU)**

In this PGU operation (schematic shown in Figure 3-left), we generally observed liquid formation in the cathode chamber due to pressure build-up. Therefore, we investigated the potential of utilizing anion exchange membrane (AEM) in the PGU in order to reduce flooding and improve reactor stability. The new schematic of the upgraded PGU assembly with AEM is shown in Figure 3-right. The upgraded PGU with AEM system consists of graphite cathode chamber with GDE and an anion exchange membrane (AEM; Sustainion® Alkaline Anion Exchange Membrane X37-50 grade RT) pressed against GDE. The remainder of the configuration was unchanged. This modification enabled an enhanced pressure balance between the cathode gas chamber and the catholyte chamber, reduced flooding, and improved reactor stability.



**Figure 3. Conceptual schematic of the PGU and upgraded PGU with the incorporation of an anion exchange membrane.**

The following discussion can also be found in our 2019 and 2021 ICES manuscript<sup>3,4</sup> but due to its importance in understanding the operation of the PGU, this text has been included here. During the operation of the PGU, the deionized (DI) water source will enter the liquid catholyte inlet port (in the catholyte chamber). Water from the solution diffuses across the proton exchange membrane (PEM; Nafion 117) to the catalyst-PEM interface at the anode. When an electrical overpotential is applied to the cell, water is converted to oxygen gas and hydrogen ions at the mixed metal oxide (MMO) anode ( $H_2O \rightarrow \frac{1}{2} O_2 + 2H^+ + 2e^-$ ). These hydrogen ions are transported from the anode-PEM (NAFION 117) interface through the membrane and across the ion exchange media (Amberlite IR120) and catholyte (DI water) to the cathode gas diffusion electrode (GDE; CeTech WIS1005) where it reacts with the available hydroperoxyl ions from the oxygen reduction reaction ( $O_2 + H_2O + 2e^- \rightarrow HO_2^- + OH^-$ ) to form hydrogen peroxide ( $HO_2^- + H^+ \rightarrow H_2O_2$ ) at the cathode's catalyst-catholyte-gas (solid-liquid-gas) interface. Attraction of molecules to the positive charge of each proton exchange through the PEM results in a flux of previously-diffused liquid water transporting across the PEM from the anode to the catholyte, this behavior is called "protonic drag". A smaller fraction of the protonic drag is forced back at a rate determined by pressure, temperature and membrane conditions. At sufficiently low current density, the net flux of water is dominated by diffusion across the PEM to react at the cathode catalyst-PEM interface. Catholyte, air, and hydrogen peroxide solution from each operating cell collects in each cell stack's internal manifold and exits each cell stack. The cell stack is powered via a dedicated power supply. The

upgraded sub-scale PGU is currently being used for testing. Iodometric titration<sup>6</sup> was implemented to measure the concentration of peroxide obtained from the PGU.

During our earlier demonstration (2019 and 2021 ICES manuscript<sup>3,4</sup>), oxygen feed gas and RO water was utilized to generate 1L per day of 2 w/w% peroxide. Discussions with NASA identified the need to examine air as a feed gas source and other lower conductivity electrolytes readily available at the ISS, including DI water. This modified PGU was further transitioned to utilize air as feed gas and deionization (DI) process treated water.

## C. Microbial Materials and Methods

### 1. Microorganisms and Growth Conditions

To best represent microorganisms found on spacecraft surfaces, two bacteria and one fungus frequently recovered from ISS surfaces were used in this study. *Staphylococcus epidermidis* and *Bacillus* species were returned on SpaceX-23, while *Aspergillus brasiliensis* was returned on Soyuz 57. Following retrieval from frozen archival storage, the bacteria were cultured on Tryptic Soy Agar (TSA, Hardy Diagnostics, Santa Maria, CA) and the fungus was cultured on Sabouraud Dextrose Agar containing 0.005% Chloramphenicol (SABC, Hardy Diagnostics).

### 2. Microbial Consortium Generation

To create a microbial consortium, fresh fungal spores from a 5-day-old plate were transferred to a fresh SABC media plate and allowed to grow for 7 days at 25 °C. Following the 7 days of growth, a 10 µL loop was gently touched to the mature fungus and then mixed into 10 mL of sterile Butterfield's phosphate buffer (PB, Weber Scientific, Hamilton, NJ). The suspension was vortexed for 1 minute to ensure the spores were well-dispersed into the buffer. The resulting concentration was  $\sim 6.4 \times 10^4$  colony forming units (CFU)/mL. A single colony from a 24-hour culture on TSA of either *S. epidermidis* or *Bacillus* sp. was inoculated into 10 mL of Tryptic Soy Broth (TSB, Hardy Diagnostics) and allowed to grow for 16 hours with shaking (150 rpm) at 35 °C. After 16 hours, 100 µL of the overnight culture was transferred to 10 mL of fresh TSB and cultured for 4 hours under the conditions previously described. Following 4 hours, the culture was centrifuged at 3500 rpm for 5 minutes, the media was removed, the cell pellet was washed twice with PB, and the cell pellet was then resuspended in PB. The optical density (OD) at 600 nm was adjusted to 0.6 for *Bacillus* sp. and 0.3 for *S. epidermidis* equating to  $\sim 6.8 \times 10^7$  CFU/mL and  $\sim 8.3 \times 10^7$  CFU/mL, respectively. For both the fungus and bacteria, the colony counts were confirmed through serial dilution and plating. The bacteria were mixed and diluted to a final concentration of  $\sim 6.4 \times 10^2$  CFU of *A. brasiliensis*,  $\sim 6.8 \times 10^3$  CFU of *Bacillus* sp., and  $\sim 8.3 \times 10^3$  CFU of *S. epidermidis*. This consortium was used for all studies.

### 3. Killing Efficacy of Hydrogen Peroxide

Faraday-generated H<sub>2</sub>O<sub>2</sub> (F H<sub>2</sub>O<sub>2</sub>) was compared to a stock provided by the NASA JSC Environmental Chemistry Laboratory (N H<sub>2</sub>O<sub>2</sub>). The following concentrations were assessed by adding 100 µL of the microbial consortium to sterile water (no treatment control) and water with H<sub>2</sub>O<sub>2</sub> at final concentrations of 1.5% F and N H<sub>2</sub>O<sub>2</sub>, and 3% F and N H<sub>2</sub>O<sub>2</sub>. An aliquot was removed for plating on TSA (Hardy Diagnostics) and incubation at 35 °C after 1, 8, 12, and 18 minutes. Following 18 minutes, the entire volume was plated on TSA (Hardy Diagnostics) and incubated at 35 °C. Colonies were counted at multiple timepoints to ensure that both the bacteria and fungus were captured. The results were expressed in percentage of growth for each sample versus collection time. Each test was performed in triplicate. The average percentage and standard deviation were calculated, and a one-way ANOVA was performed between each treatment at each time point in comparison to the blank control.

### 4. Wipe Disinfection Evaluation

- Wipe Generation

To generate wipes for testing, 2 mL of either F H<sub>2</sub>O<sub>2</sub>, N H<sub>2</sub>O<sub>2</sub>, or sterile water was added to a cloth wipe (Wypall X60, Kimberly-Clark, location). These 3% H<sub>2</sub>O<sub>2</sub> and wipes were compared to the sterile water wipe, as well as the current ISS housekeeping disinfectant wipe (PREempt® Wipes, Virox Technologies, Inc., ON, Canada).

- Coupons

Stainless steel coupons (1" × 1.5") were washed, wiped with ethanol, and sterilized by autoclaving prior to use in these studies. One hundred µL of the previously-described consortium was added directly to the top of the coupon and allowed to dry for 40 minutes within a biological safety cabinet. Once dry, the coupons were wiped with either the sterile water control wipe, the 3% F or N H<sub>2</sub>O<sub>2</sub> wipe, or the current ISS disinfection wipe. Wiping was performed by a single individual to control applied pressure. Wiping motion and time was also controlled. Following wiping, the

coupon was transferred into a 50 ml conical tube containing 15 mL of PB. The tube was sonicated for 10 minutes, and the 15 mL was filtered through a TSA cassette (Millipore Sigma, Burlington, MA). The cassettes were incubated at 35 °C, and colonies were counted at multiple timepoints to ensure capturing both bacteria and fungus. For each condition, triplicate coupons were used. The results are expressed in terms of the percentage of growth in comparison with the sterile water wipes.

- Environmental Surfaces

Seven surfaces (see Table 3) within Building 21 at the NASA Johnson Space Center were disinfected with either the current ISS wipe or a wipe treated with 3% F H<sub>2</sub>O<sub>2</sub> as previously described. Wiping was completed by the same individual using the same surface area, wiping motion, and wiping time. After allowing the areas to completely dry, they were sampled using the Microbiology Laboratory's standard operating procedure for surface sampling. Briefly, a 25 cm<sup>2</sup> area of the disinfected surface was swabbed and the swab was transferred to 3 mL of TSB. Following vortexing, the TSB was plated onto TSA and SABC and incubated. Colonies were counted after 48 hours and 5 days for bacteria and fungus, respectively.

### III. Results and Discussion

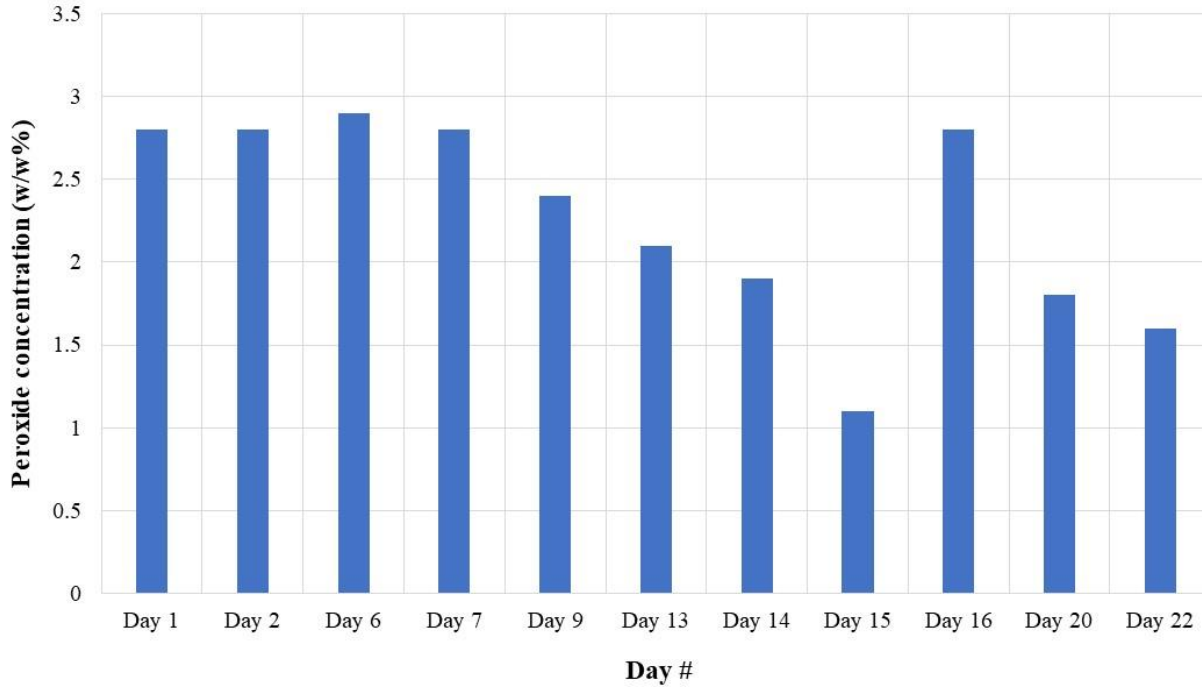
#### A. Peroxide Generation Unit Demonstration

##### 1. Sub-scale PGU

The sub-scale PGU was capable of continuously producing ~3 w/w% hydrogen peroxide over ~20 days with air as feed gas and DI water as catholyte. The peroxide concentration consistently reached ~1 w/w % after 5 hr and ~3 w/w % after 24 hr of operation at 0.4A. The following conditions were used for the evaluation:

- Duration – 22 days
- Pass – Multi pass
- Catholyte – 50 mL DI water @ 25 mL/min
- Gas Feed source – Compressed Dry Air
- Gas Flow rate- 30 mL/min
- Pressure balance (BPR) – 1 psi
- Cell arrangement: Vertical Cell Alignment
- Cathode: CeTech W1S 1009 GDE
- AEM: Sustainion® Grade X37-50 RT anion exchange membrane
- Ion exchange beads – Amberlite IR 120 Hydrogen form
- Volume of Catholyte with beads: 3 mL
- PEM: Nafion 117 cation exchange membrane
- Anode: Ti plate with MMO DSA
- Applied Current: 0.4 A
- Threshold Voltage: 30 V
- Water circulation in Anode: No
- Time 24 h

After each trial the PGU was rinsed with DI water. Then the air and liquid flow were turned off (keeping DI water in the cell) at 1 PSIG of back pressure until initiation of the next trial on a new aliquot of solution. Iodometric titration was performed after each trial to determine the peroxide concentration. The results for each of the stability trials is summarized in Figure 4. For Day 15, Trial 8, the gas flow meter was stuck at ~10mL/min during the night and hence lower peroxide concentration was achieved. The efficiency of the PGU began to decrease after ~20 days operation. We are currently trying to identify the mechanism of this degradation.



**Figure 4:** Results of durability tests

### 2. Peroxide product stability

The stability (shelf-life) of one sample of peroxide produced with DI water and air was further studied. The produced peroxide was stored in plastic containers. The concentration of peroxide after storing over the specified period was determined by iodometric titration, as shown in Table 2. The data indicates the stability of electrochemically generated peroxide after three weeks of storage.

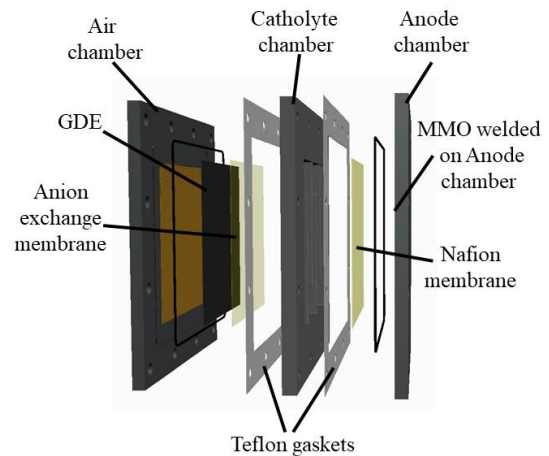
**Table 2.** Stability of peroxide solution stored in plastic container over 22 days

Peroxide concentration w/w% measured by titration			
Day 1	Day 8	Day 15	Day 22
2.9	2.8	2.8	2.8

### 3. Alpha-scale PGU

Utilizing the sub-scale PGU platform, we performed scaling analysis in order to design an alpha scale PGU that can produce 1 L of 3 w/w%  $H_2O_2$  in DI water. We then assumed that the electrode scales linearly and left some scaling room to enable the system to produce higher quantities of peroxide in the future. Based on these estimates, the alpha scale PGU system was determined to have a planar working area of about 125 cm<sup>2</sup> to produce 1 L of 3 w/w%  $H_2O_2$  in DI water per day. Accordingly, we designed and built an alpha scale PGU system (Figure 5), whose components consisted of:

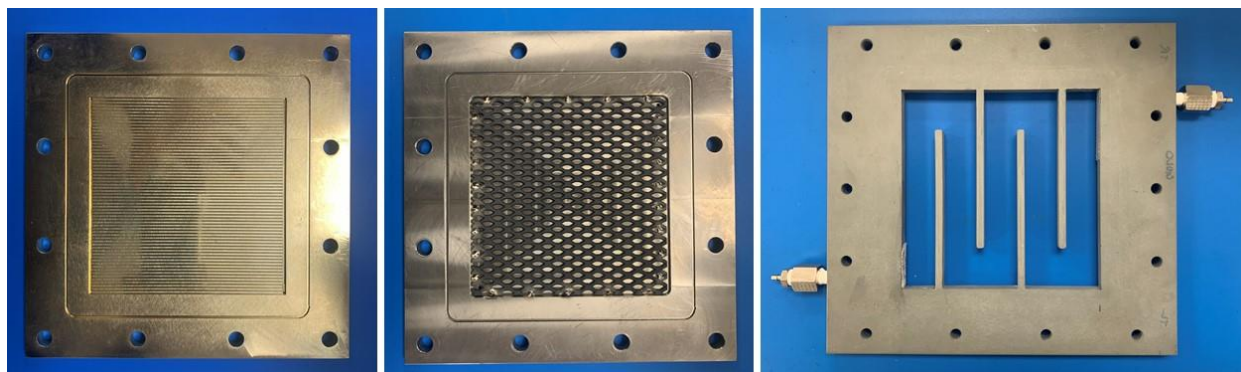
- Air/oxygen chamber which is gold plated SS316 with a central 5" x 5" flow field (Figure 6 - left)
- PVC catholyte chamber with a 5" x 5" central working area with serpentine flow field channel (Figure 6 - center)
- Anode chamber which is titanium block with a central 5" x 5" MMO mesh (Figure 6 - right)



**Figure 5.** Exploded view of the alpha-size reactor.



The exploded view of this alpha-scale is shown in Figure 5, which is currently being used to demonstrate the potential of generating 1 L per day of 3 w/w% hydrogen peroxide.



**Figure 6.** Air/oxygen chamber gold plated SS316 with a central 5'' x 5'' flow field (left); anode chamber titanium block with a central 5'' x 5'' MMO mesh (center); and PVC catholyte chamber with serpentine flow field design (right)

Initial process optimization in the alpha-scale reactor using oxygen as feed gas were performed using the following processing conditions:

- Pass – Multi pass
- Catholyte – 1 L DI water @ 30 mL/min
- Gas Feed source – Oxygen UHP300
- Gas Flow rate- 100 cc/min
- Pressure balance (BPR) – 0.5 psi
- Cell arrangement: Vertical Cell Alignment
- Cathode: CeTech W1S 1009 GDE
- AEM: Sustainion® Grade X37-50 RT anion exchange membrane
- Catholyte chamber: Serpentine flow field
- Ion exchange beads – Amberlite IR 120 Hydrogen form
- Volume of Catholyte with beads: 80-100 mL
- PEM: Nafion 117 cation exchange membrane
- Anode: Ti plate with MMO
- Applied current: 2.5 A
- Threshold Voltage: 30V
- Water Circulation in Anode: No

Iodometric titration measured after 24 h run indicates the peroxide concentration to be ~3 w/w%. The hydrogen peroxide generated using the alpha-scale reactor was sent to NASA for microbial property control characterization, which is described below. In future studies, we plan to continue utilizing DI water to generate peroxide in the alpha-scale PGU and assess the sensitivity of the PGU to air being a feed source for replacing oxygen. Additionally, we will continue to optimize the system to improve its rate of peroxide production and improve its durability and lifetime.

## **B. Disinfection Demonstration**

The delivered hydrogen peroxide solutions were analyzed by the JSC Environmental Chemistry Laboratory (ECL) using a titrimetric method with a reporting limit of 0.003%. Faraday-generated  $\text{H}_2\text{O}_2$  (F  $\text{H}_2\text{O}_2$ ) was compared to a stock provided by the NASA JSC ECL (N  $\text{H}_2\text{O}_2$ ). The F  $\text{H}_2\text{O}_2$  sample was found to have a concentration of 3.77 w/w%, while the N  $\text{H}_2\text{O}_2$  sample was found to have a concentration of 3.00 w/w%.

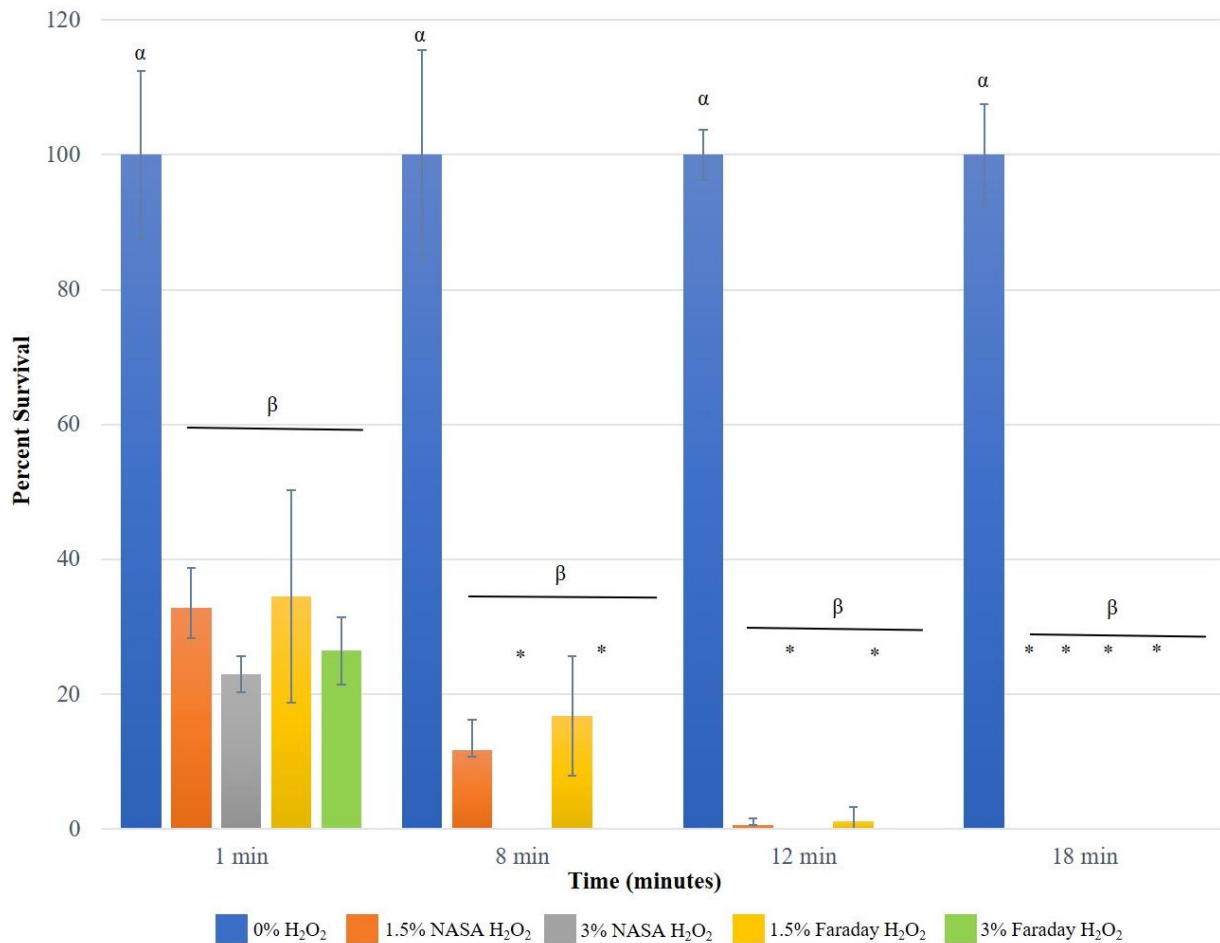
### *1. Killing Efficacy of Hydrogen Peroxide*

After one minute of exposure at a concentration of 1.5%, 66% and 67% of microbial consortium treated with Faraday and NASA hydrogen peroxide, respectively, were dead, as opposed to 100% viability of the nontreated control



(Figure 7). When the contact time increased to eight minutes, the efficacy of disinfection was enhanced to 83% and 88% by the Faraday and NASA hydrogen peroxide, respectively. At 12 and 18 minutes, the killing efficacy reached 99% and 100%, respectively for both the Faraday and NASA hydrogen peroxide (Figure 7). As such, no viable microbial cells survived to the 18-minute time point as determined by plating the remaining test solution. In the case of 3% hydrogen peroxide, after the first minute, 74% and 77% of the consortium had been killed by the Faraday and NASA hydrogen peroxide, respectively. With the increasing contact time, at eight minutes, neither viable bacteria nor fungus could be recovered (Figure 7).

The percentage of hydrogen peroxide evaluated, 1.5% and 3%, here are shown to be effective as a disinfectant toward common surface-contaminating bacteria and fungus. Additionally, these findings correlate to previous assessments on spaceflight isolates<sup>7</sup> and confirm that, at 3%, hydrogen peroxide exposure results in a nonviable consortium with under 8 minutes of contact time. By increasing the dwell time to 12 minutes, a lower concentration of hydrogen peroxide is effective. Additionally, there was no significant difference observed between the Faraday generated hydrogen peroxide and that provided by the JSC ECL. This demonstrates the utility of the generated hydrogen peroxide toward effective killing of microorganisms.

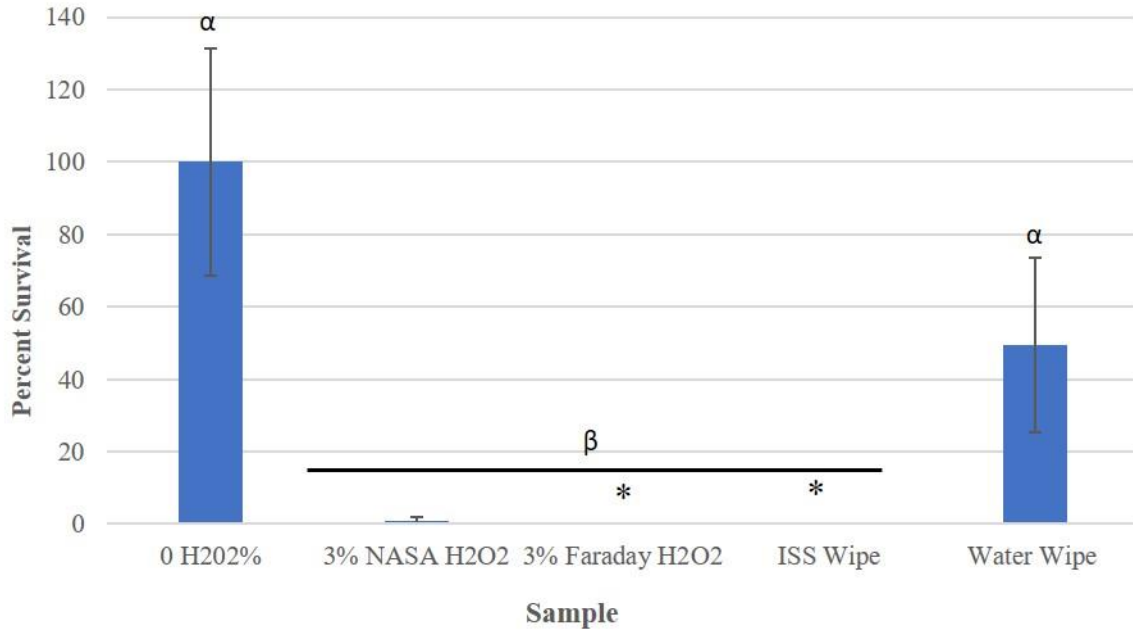


**Figure 7. Percentage survival of microbial consortium following hydrogen peroxide challenge.** Standard deviation was calculated from triplicate samples. (\*) represents a value of zero (no growth observed). One-way ANOVA was calculated to determine if a statistically significant difference existed at each time point denoted and (β) denotes a statistically significant difference (p value < 0.05) as compared to the no treatment control denoted by (α).

## 2. Coupon and Environmental Surface Disinfection Evaluation

Mechanical wiping performs some microbial removal. However, a hydrogen peroxide wipe is more effective than a water-wetted wipe at achieving surface disinfection. The current ISS disinfection wipe and the wipe wetted with Faraday hydrogen peroxide both resulted in the inability to recover any of the microbial consortium, as compared to

the water-wetted wipe where only a 51% reduction was noted (Figure 8). Parallel effectiveness was observed following the disinfection of environmental surfaces with the ISS wipe and 3% Faraday wipe, as neither bacteria nor fungi were recovered (Table 3). Taken together, the simulated consortium applied to coupons and the testing of environmental surfaces following wiping demonstrates the high effectiveness of the Faraday-generated hydrogen peroxide as a disinfectant. Furthermore, based on this work, there is no significant difference in disinfecting ability between a wipe wetted with Faraday hydrogen peroxide and the current ISS wipe. The Faraday generated hydrogen peroxide would be an efficient alternative for disinfection of ISS surfaces.



**Figure 8.** Percentage survival of microbial consortium on coupons following wiping. Standard deviation was calculated from triplicate samples. (\*) represents a value of zero (no growth observed). One-way ANOVA was calculated to determine if a statistically significant difference existed at each time point denoted and (β) denotes a statistically significant difference (p value < 0.05) as compared to the no treatment control denoted by (α).

**Table 3.** CFU Recovered Following Disinfection of Environmental Surfaces

	ISS Wipe		Faraday 3% Wipe	
	Bacteria Recovered (CFU/25 cm <sup>2</sup> )	Fungi Recovered (CFU/25 cm <sup>2</sup> )	Bacteria Recovered (CFU/25 cm <sup>2</sup> )	Fungi Recovered (CFU/25 cm <sup>2</sup> )
Toilet Seat	0	0	0	0
Lunch Table	0	0	0	0
Lab Bench space	0	0	0	0
Restroom Counter	0	0	0	0
Cubicle 1112D	0	0	0	0
Huddle Room 1116	0	0	0	0
Breakroom Counter	0	0	0	0

#### IV. Conclusions

A sub-scale peroxide generation unit was upgraded to demonstrate the potential of *in-situ* on-demand generation of hydrogen peroxide disinfecting solution utilizing on-board life support components of air and DI water. The data included herein demonstrated that the sub-scale PGU was able to maintain operation over 20 days for generating ~3 w/w% hydrogen peroxide disinfecting solution. The sub-scale PGU platform was utilized to design and build an alpha scale PGU to generate at least 1L of ~3 w/w% H<sub>2</sub>O<sub>2</sub> in a day using life support DI water supplies and pure oxygen.

Microbial control property characterization demonstrates the high effectiveness of the Faraday-generated hydrogen peroxide as a disinfectant. The electrochemical peroxide generation system would be an efficient alternative with the disinfectant being generated on demand and in-situ for disinfection of spacecraft surfaces.

## V. Future Demonstration/Prospects

In addition to being an integral component of long-term life support on NASA crewed space missions, the PGU integrated with a concentrator technology has the potential to produce high concentration peroxide for propulsion application. Terestrially, the PGU can serve as an alternative method for synthesis of commercial hydrogen peroxide for on-demand onsite disinfection of process waste streams and/or field remediation systems.

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